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Published in:
Proceedings of the 22nd International Conference on Miniaturized Systems for Chemistry and Life Sciences

Publication date:
2018

Document Version
Publisher's PDF, also known as Version of record

Optimizing supercritical angle fluorescence structures in polymer microfluidic chips for highly sensitive pathogen detection

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ABSTRACT
We report an in-depth analysis and a fabrication method to precisely produce micro-sized arrays of supercritical angle fluorescence optical structures in disposal microfluidic polymer chips. This technique can be used for industrially massive production of disposal microfluidic polymer chips for pathogen detections with application in food safety and clinical diagnosis. For a demonstration, the chip is used to successfully detect E. coli with the limit of detection of 3.37×10^2 copies.

KEYWORDS: microfluidics, pathogen detections, diagnosis, polymer injection molding, fluorescence, PCR.

INTROUCTION
Excited fluorescent molecules positioned at the interface of two dielectric media emit a large amount of their radiation into the higher refractive index medium [1], (Fig. 1a). The angle at which the significant emission can be observed in the denser medium is called supercritical angle since it is above the angle of total internal reflection, i.e. the critical angle of the two media. The fluorescence observed in this context is hence called supercritical angle florescence (SAF). The optical structures in which SAFs are observed are called SAF structures (Fig. 1b). Our group developed a technique to fabricate micro-sized SAF arrays on which a solid-phase polymerase chain reaction (sp-PCR) for multiplexed detection of pathogens was performed with a high sensitivity and a low volume of reagent [2]. In the fabrication step of micro SAF arrays, the optimization of the SAF structures has however not yet been reported. There must be a relation between the critical angle and the height of the SAF structure so that the maximum fluorescence intensity can be observed, hence the optimal limit of detection (LOD). Here, we present for the first time an in-depth analysis and experimental results for optimization of the supercritical angle fluorescence (SAF) structures in microfluidic chips fabricating from the combination of micro milling and polymer injection molding techniques in application on highly sensitive detection of pathogens.

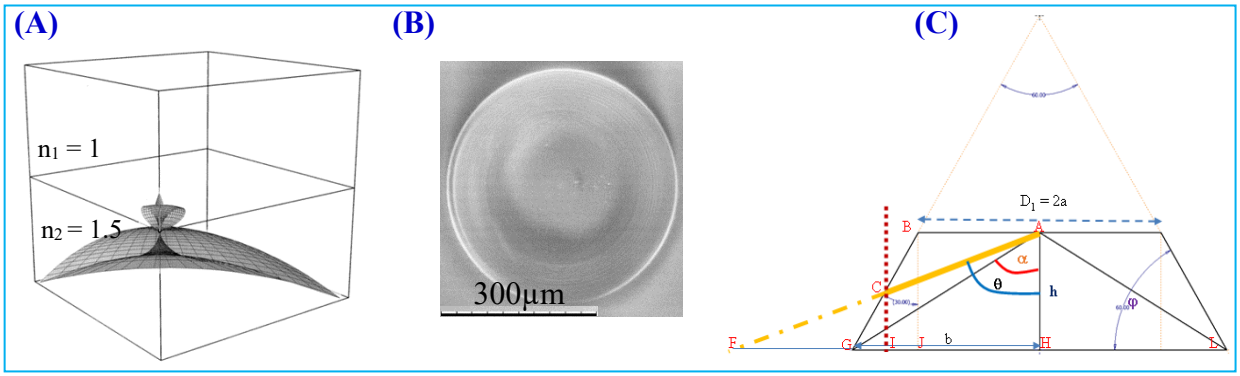
THEORY


Figure 1: **(A)** *Large amount of the radiation emitted into the higher refractive index medium [1].* ***(B)*** *a typical micro-sized SAF structure fabricated using polymer injection molding in our work;* ***(C)*** *a schematic model of a SAF structure in which the critical angle (θ), the side angle (φ) and the radius a play important roles to determine its height h.*

Our calculation leads to the formula for determination of the optimal height (h) of a SAF structure, see Fig. 1C for nomenclature:

$$h = a \times \tan(\varphi) / [(\tan(\theta) \times \tan(\varphi) - 1] \qquad (1)$$

EXPERIMENTS
Together with theoretical modeling, we experimentally fabricate microarrays of SAF structures with different heights varying from zero to 313 µm in Cyclic Olefin Copolymer (COC) microfluidic chips. The solid-phase PCR is then conducted on these chips to observe the best LOD that is corresponding to the optimal height of the SAF structure. For a demonstration, the chip is then used to detect E. coli.

RESULTS AND DISCUSSION

The micro-milling aluminum insert using in polymer injection molding is shown in Fig. 2A. Fig. 2B shows the SEM images of different micro SAF structures (varying of heights) in an array in the polymer injection-molding chip. SEM images with same magnification of individual SAF structures having different heights are shown in Fig. 2E & 2F. Accordingly, the heights are plotted in Fig. 2C.

The highest fluorescence intensities are obtained at SAF structures with 159 µm height when using the milling tool DIXI 7006 (60°) (as shown in Fig. 2D).

From eq. (1), with $\theta = 40.8^{o}$, $\varphi = 60^{o}$, a = 50 µm (measured from SEM images, Fig. 2F), we obtain the theoretical height of SAF structure is h = 174 µm. The results shown in fig. 2D is consistent with the estimated height from eq. (1) as the fluorescent intensity, which increased with the height of the SAFs, reaches the peak on SAF structure at 159 µm. When SAF structure is higher than 174 µm, in this experiment they are 209 µm and 313 µm, the fluorescent intensities were gradually decreased. This can be explained by the fact that the fluorescent intensity can be disturbed by optical noise due to increasing of surface area and surface roughness at the side walls if the SAF structures are too high. When the height of the SAF structure is too low, we lose the observation signal due to reaching the limit of the critical angle i.e. total internal refection.

Accordingly, the limit of detection (LOD) is at 5.42×10^{4} molecules (Fig. 2G). The chips with optimal SAF structures are used to successfully detect E. coli with the limit of detection of 3.37×10^{2} copies (shown in Fig. 2H).

These results especially are of interest for application in hypersensitive pathogen detection as well as assist the design of devices for point of care application. The findings on the height optimization of SAF structures also advance our understandings of SAF detection technique and provide insights in developing of fluorescent microscopy.

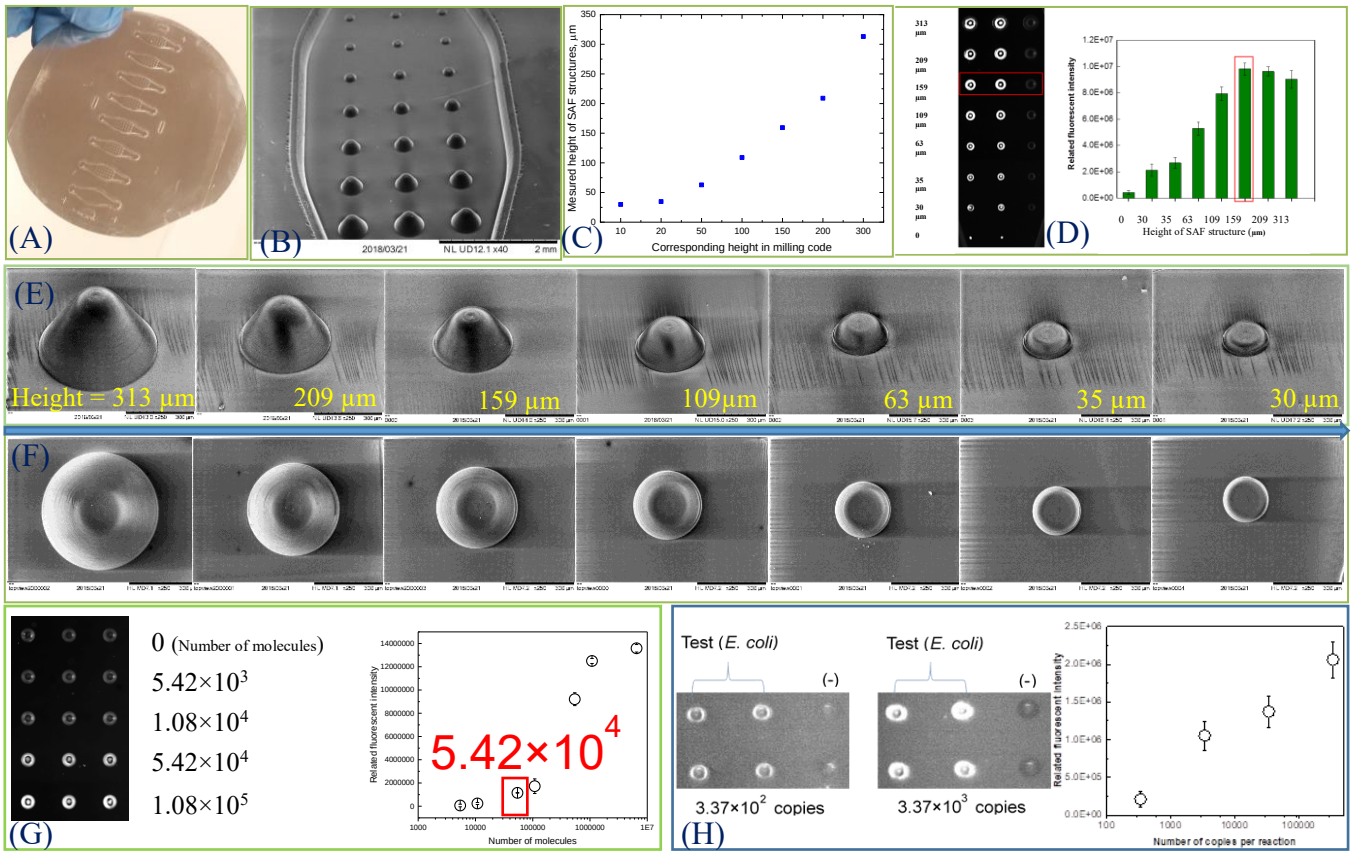


Figure 2: **(A)** *Micro-milling Aluminum insert used for polymer injection molding.* ***(B)*** *SEM images of an micro-sized SAF array fabricated in this work.* ***(C)*** *Graph of measured heights of each SAF structure using Dektak.* ***(D)*** *Fluorescent intensity versus height of the SAF structure. SEM images under same magnification of individual SAF structures with different heights measured from the same chamber and chip: 45-degree views* ***(E)****, top views* ***(F)****.* ***(G)*** *Fluorescent limit of detection when using SAF structure with height of 159 µm.* ***(H)*** *solid-phase PCR on the chip with 159 µm height, E. coli is the target pathogen.*

References: **[1]** *Appl. Opt. 38, 724-732 (1999)*; **[2]** *Lab Chip, 2015,15, 2445-2451.*